89033.

International Conference

Heavy Metals
in the
Environment

Volume One

Geneva - September 1989

Editor: J-P Vernet

© CEP Consultants Ltd 1989

Published by CEP Consultants Ltd, 26–28 Albany St, Edinburgh EH1 3QH, UK

Printed in Great Britain by Page Bros (Norwich) Ltd.

90-06466/

INHIBITORY AND STIMULATORY EFFECTS OF Cu, Cd, Zn, Hg, AND Se ON MICROBIAL PRODUCTION OF METHYL MERCURY IN SEDIMENTS

T. A. Jackson*

ABSTRACT

Laboratory experiments demonstrated that Cu, Cd, Zn, Hg, and Se may either inhibit or promote microbial methylation of Hg in lake sediments. A progressive increase in metal or Se concentration caused alternating decreases and increases in methyl Hg production, along with corresponding variations in other indicators of microbial activity (Eh, pH, and CO_2 and CH_4 levels), in mud from metal-polluted lakes. The results are attributed to alternating suppression of sensitive microbial species and stimulation of tolerant ones. Mud from an unpolluted lake showed a steady decline in methylating activity but no resurgence, implying absence of tolerant species. The study showed that toxic pollutants may have complex, variable, and selective effects on microbial activities owing to ecological succession in the microbial community, and that Se may not be totally reliable as an environmental detoxifying agent in cases of Hg pollution.

INTRODUCTION

Micro-organisms in sediments vary widely in their requirements, limits of tolerance, and mutual interactions. The dominant species at any given time are those which are best adapted to the prevailing conditions, and a change in environment such as the introduction of a toxic pollutant may cause other, better adapted species to replace them, altering the character and activities of the microflora. This paper deals with effects of heavy metals and selenium on the microbial transformation of inorganic mercury (Hg) into methyl mercury (CH_3Hg^+) in lake sediments. CH_3Hg^+ is of major concern because it is highly toxic, undergoes biomagnification up the food chain, and accumulates in fish. Numerous free-living microbial species synthesise or decompose CH_3Hg^+. The net rate of CH_3Hg^+ production from a given quantity of inorganic Hg depends on environmental variables, as these determine the species composition and activities of the microflora as well as the availability of the inorganic Hg. Little is known about the effects of toxic pollutants on CH_3Hg^+ production, but previous work by the writer yielded evidence for selective inhibition of some methylators and stimulation of others (ref 1; cf ref 2). The present paper, which represents an extension of that research, treats the results of experimental study of effects of Cu, Cd, Zn, Hg, and Se on CH_3Hg^+ production in various lake muds.

MATERIALS AND METHODS

Mud samples were dredged from (1) Clay Lake (CL), Ontario, (2) Tyrell Lake (TL), Saskatchewan, and (3) East Mynarski Lake (EML), Manitoba in the Boreal forest zone of Canada. CL is polluted with Hg from a chlor-alkali plant, and TL is polluted with several metals, notably Cu, Cd, and Zn, from a smelter, but EML is "unpolluted." The mud was analysed chemically, and replicate homogenised portions of it were enriched with nutrients (plant fragments) and suspended in equal volumes of water containing a fixed concentration of dissolved $HgCl_2$ with varying

*National Hydrology Research Institute, 11 Innovation Blvd., Saskatoon, Saskatchewan S7N 3H5, Canada.

concentrations of $CuCl_2$, $CdCl_2$, $ZnCl_2$, or Na_2SeO_3 (or varying $HgCl_2$ concentrations); the slurries were then incubated in the dark for 7 days at 20-25°C under N_2 in stoppered flasks with valves for sampling head gas. Slurries with no added test substance were used as controls. The slurries were either unbuffered or buffered with $CaCO_3$ or Tris to maintain a constant pH close to 7. The flasks and their contents (except for the mud) were sterilised before incubation. Starting conditions were assessed by means of "day zero" controls. After incubation the head gas was analysed for CO_2 and CH_4 by gas chromatography (GC), and the pH and Eh of the slurries were measured. The CH_3Hg^+ content of each slurry was determined by solvent extraction generally followed by analysis of the extract by GC; in experiments employing CL mud CH_3Hg^+ was estimated as total "organic Hg" by flameless atomic absorption spectrophotometry (AAS). Analysis of a suite of sample extracts by both GC and AAS led to the conclusion that CH_3Hg^+ and organic Hg, being strongly correlated ($r = 0.964$; $P < 0.001$), are essentially equivalent. The experimental and analytical techniques are described in detail elsewhere (refs 1, 2, 3).

RESULTS AND DISCUSSION

The CH_3Hg^+ production rate in EML sediment decreased progressively with increasing Cu, Cd, or Zn concentration (fig 1A). In the presence of Cu this decline was preceded by a slight rise in CH_3Hg^+ level, but the trends are practically featureless, indicating that the effect of the metals is almost uniformly inhibitory. In contrast, CH_3Hg^+ production in CL and TL sediments showed major oscillations in response to increasing Cu, Cd, Zn, or Se concentration (figs 1B, 1C, 1D). The patterns of variation were reproducible and were essentially the same for buffered and unbuffered slurries even though the absolute values differed somewhat. The CH_3Hg^+ yield often decreased initially, as in EML sediment, but then rose to form a large maximum or succession of maxima which commonly exceeded the CH_3Hg^+ level in the control, and finally resumed its decline at the highest concentrations of the test substance. Analytical data for mud samples from several metal-contaminated lakes near TL showed a strikingly similar relationship between CH_3Hg^+ and total Cd content (ref 1, fig 14.4a), suggesting that the phenomena observed in the experimental systems also occur under field conditions. The TL data display close correspondence between variations in CH_3Hg^+ production and variations in other microbial functions, signifying that the changes in CH_3Hg^+ level were caused by major changes in the nature and biochemical activities of the microflora. Thus, the peaks in CH_3Hg^+ yield coincide with maxima in CO_2 level and pH and minima in Eh (figs 1C, 1D). However, there was no cause and effect relationship between CH_3Hg^+ production and pH, as the same variations in CH_3Hg^+ level were observed when a buffer was used. In the presence of sediment from the same sampling site, all test compounds gave similar patterns of variation but differed in their quantitative effects per unit molar concentration; for instance, Zn had a less toxic effect (fig 1A) and a greater stimulatory effect (fig 1B) than Cd or Cu. The degree of inhibition or promotion of methylation depended on certain properties of the element, such as its electronegativity, the "excess polarising strength" of its cation, or the standard entropy of its sulfide, suggesting that unfavourable effects result from strong covalent binding of active sites (such as -SH groups) of enzymes.

In CL mud the CH_3Hg^+ yield tended to increase, as expected, with rising inorganic Hg level, but superimposed on this trend was a succession of maxima and minima similar to those produced by Cu, Cd, Zn, and Se. Very little CH_3Hg^+ was generated until the inorganic Hg concentra-

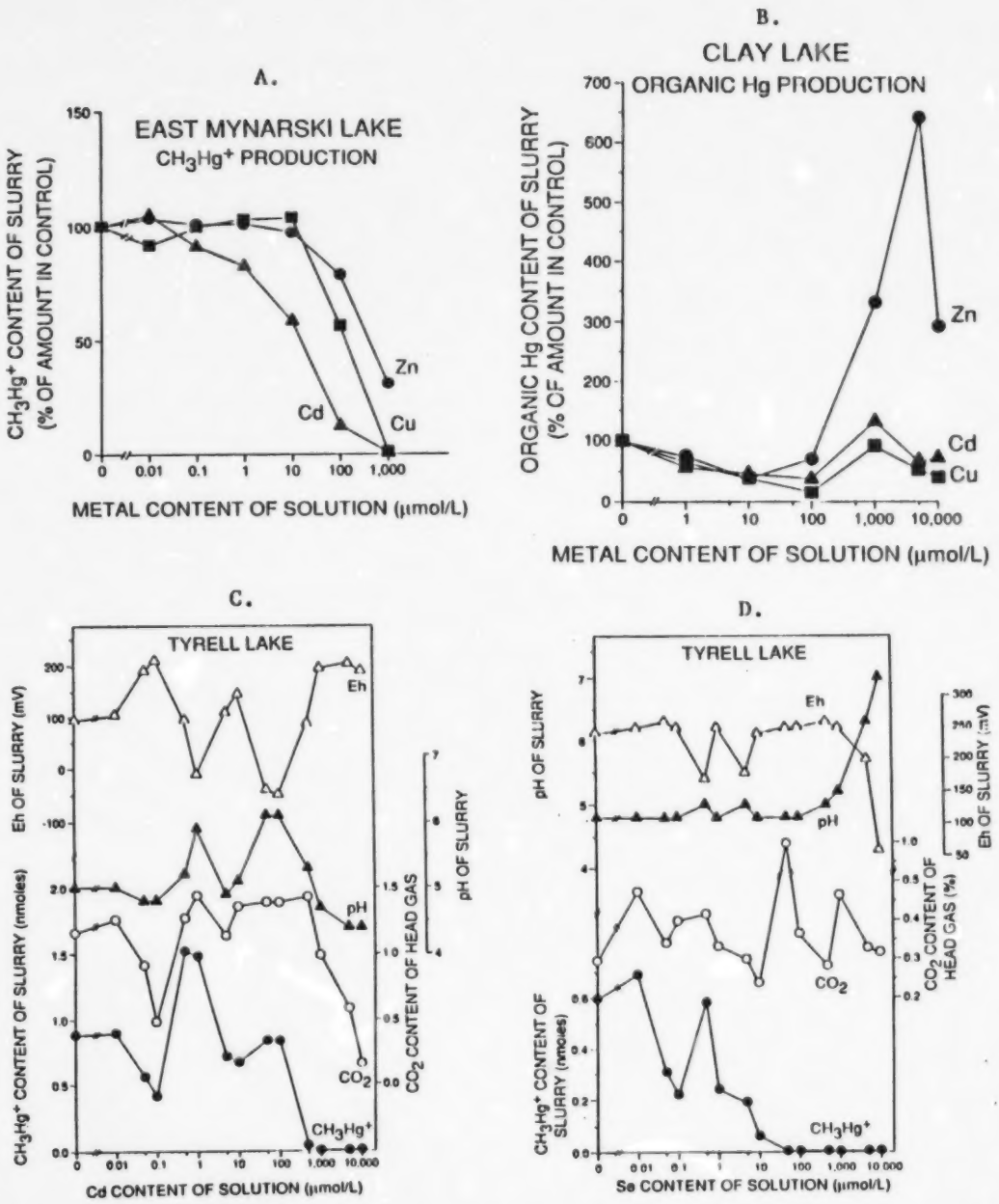

Fig. 1. Effects of Cu, Cd, Zn, and Se on CH_3Hg^+ production and related indices of microbial activity in lake sediments. (Source of data in fig 1B: ref 1).

tion exceeded 1,000 μmol/L, when the CH_3Hg^+ level abruptly rose to form a large peak coinciding with a peak in CH_4 production and a decline in both pH and CO_2 level (fig 2). This suggests a major upsurge in methylation rate owing to an upsurge in the growth of Hg-methylating CH_4 bacteria.

The zigzagging series of CH_3Hg^+ minima and maxima observed in CL and TL mud are attributed to ecological succession in the microbial community - specifically alternate suppression of metal- and Se-sensitive methylating microbes and stimulation of tolerant ones. With a rise in metal or

Se concentration the more sensitive species were eliminated, causing a fall in methylation rate; but beyond a critical threshold concentration certain relatively tolerant species, which had been kept in check by competition with the sensitive ones, were able to grow, initiating a resurgence of methylating activity. With a further rise in metal or Se level, these species, in turn, were inhibited and replaced by still more tolerant species. The corresponding fluctuations of other indices of microbial activity support this interpretation and prove the occurrence of important successional changes in the nature and activities of the microflora. The apparent absence of tolerant species in EML mud is understandable: In an unpolluted environment such as EML organisms are not under pressure to evolve adaptations to toxic pollutants.

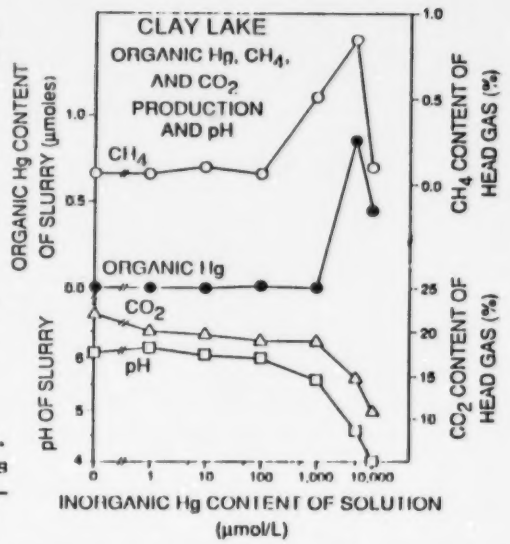

Fig. 2. Effects of inorganic Hg on CH_3Hg^+ and CH_4 production and other indices of microbial activity. (Source of CH_3Hg^+ and CH_4 data: ref 1).

The results of the study demonstrate that toxic pollutants, by selectively inhibiting some types of microbe and fostering the activities of others, may have dramatic, variable, and unpredictable effects on natural microfloras. As the members of a microbial community typically vary widely in their ecological requirements, limits of tolerance, and activities, and interact with each other in various ways ranging from competition and antagonism to mutualism, the complexity of the direct and indirect effects of pollutants can well be appreciated - especially if there are synergistic or antagonistic interactions between two or more pollutants. These effects may have far-reaching significance for aquatic ecology.

Finally, the results have implications for the environmental role of Se. Se has aroused interest because of its ability to detoxify Hg and other heavy metals and hinder the accumulation of Hg by the higher organisms of aquatic food chains, and it has even been suggested that Se could be utilised to ameliorate the impact of Hg pollution in natural waters (ref 4). Nevertheless, the findings reported here show that Se may not be altogether reliable, at least as far as its effects on Hg methylation are concerned. Its net effect may well vary with Se level and environment.

ACKNOWLEDGMENTS

The research was funded by the Government of Canada. The technical work was done by K. Supeene, C. Baron, E. Thomson, and K. Parkkari.

REFERENCES

1. T A Jackson, in J Nriagu (ed), Environmental Impacts of Smelters (John Wiley & Sons, New York, 1984), p 551.
2. T A Jackson, Applied Organometallic Chem 3, 1 (1989).
3. T A Jackson, Can J Fish Aquat Sci 45, 97.
4. M A Turner and J W M Rudd, Can J Fish Aquat Sci 40, 2228 (1983).